Tohoku J. Exp. Med., 2011, **225**, 225-234

Review

Transport of Nanoparticles through the Placental Barrier

Vytautas Kulvietis,[1,2] Violeta Zalgeviciene,[3] Janina Didziapetriene[1,3] and Ricardas Rotomskis,[1,2]

[1]Biomedical Physics Laboratory, Institute of Oncology, Vilnius University, Vilnius, Lithuania
[2]Biophotonics group of Laser Research Center, Faculty of Physics, Vilnius University, Vilnius, Lithuania
[3]Faculty of Medicine, Vilnius University, Vilnius, Lithuania

Nanoparticles (NP) are organic or inorganic substances, the size of which ranges from 1 to 100 nm, and they possess specific properties which are different from those of the bulk materials in the macroscopic scale. In a recent decade, NP were widely applied in biomedicine as potential probes for imaging, drug-delivery systems and regenerative medicine. However, rapid development of nanotechnologies and their applications in clinical research have raised concerns about the adverse effects of NP on human health and environment. In the present review, special attention is paid to the fetal exposure to NP during the period of pregnancy. The ability to control the beneficial effects of NP and to avoid toxicity during treatment requires comprehensive knowledge about the distribution of NP in maternal body and possible penetration through the maternal-fetal barrier that might impair the embryogenesis. The initial in vivo and ex vivo studies imply that NP are able to cross the placental barrier, but the passage to the fetus depends on the size and the surface coating of NP as well as on the experimental model. The toxicity assays indicate that NP might induce adverse physiological effects and impede embryogenesis. The molecular transport mechanisms which are responsible for the transport of nanomaterials across the placental barrier are still poorly understood, and there is a high need for further studies in order to resolve the NP distribution patterns in the organism and to control the beneficial effects of NP applications during pregnancy without impeding the embryogenesis.

Keywords: barrier; embryogenesis; nanoparticles; nanotoxicology; placenta
Tohoku J. Exp. Med., 2011, **225** (4), 225-234.

Nanoparticles (NP) are a class of organic or inorganic substances with the size range of 1-100 nm, which may form naturally, be produced as a waste product by human activity (automobile exhaust gases or emissions of power plants) or specifically engineered for industrial or medical purposes. Natural ecosystems have been exposed to fine and ultrafine particles throughout their history, but the industrial revolution dramatically changed sources, doses, and types of NP. Nanotechnology is a rapidly developing and expanding field leading to an increase of engineered NP with conceptually new physical and chemical properties, which might induce novel effects in biological systems (Hardman 2006; Gupta 2007). Therefore, comprehensive knowledge about possible physiological effects of NP is crucial independently on whether NP exposure is intended or not. Some nanomaterials such as titanium dioxide (TiO_2) or silica nanoparticles have been already used in cosmetics, food, electronics and medicine (Yamashita et al. 2011). The growing number of commercial products and the expansion of NP application areas raise concerns about NP accumulation, long-term retention in an organism and subsequent toxic effects.

Humans are more sensitive to toxic materials in prenatal stages than in mature age and scientific studies have shown that chemicals in maternal surroundings (air, food, water) can induce pregnancy complications and embryotoxicity (Yamashita et al. 2011). The potential fetal toxicity first of all depends on the passage of particles from the maternal organism which is mainly determined by the placental barrier in the case of mammals. The NP penetration into the conceptus is becoming an important factor in veterinary and medicine as NP are actively developed as agents for optical and magnetic imaging, drugs and vaccines for both animals and humans (Adair 2009). Molecule penetra-

Received July 8, 2011; revision accepted for publication October 6, 2011. doi: 10.1620/tjem.225.225
Correspondence: Vytautas Kulvietis, 104 room, Biomedical Physics Laboratory, Institute of Oncology, Vilnius University, P. Baublio 3b, Vilnius LT-08406, Lithuania.
e-mail: vytautas.kulvietis@vuoi.lt
Ricardas Rotomskis, Biomedical Physics Laboratory, Institute of Oncology, Vilnius University, P.Baublio 3b, Vilnius LT-08406, Lithuania.
e-mail: ricardas.rotomskis@vuoi.lt

tion across the placental barrier limits applications of some conventional drugs during pregnancy, and nanotechnology might offer solutions to this problem by developing drug-NP conjugates that would accumulate in specific targeted tissue but would not be transported to fetus because of the size of the complex (Menjoge et al. 2011). Such nanotechnological approaches open new frontiers in veterinary and medicine, but the in-depth understanding of physiological processes remains of crucial importance before developing and applying NP during pregnancy.

This review focuses on NP passage through the maternal-fetal junction by analyzing the structure and functions of this barrier in the perspective of NP applications. The article surveys and analyzes current reports on transplacental NP transport using in vivo and ex vivo models. Possible adverse effects of NP applications on embryogenesis are also discussed.

Genesis of maternal-embryonic barrier

Molecule passage to embryonic tissues first of all depends on the stage of embryogenesis because of the essential changes of the physiology of the maternal-fetal barrier during pregnancy. Generally, there are some critical periods during embryogenesis when the embryo is most sensitive to exposure to toxic materials. Classical critical periods of the fetus formation include implantation, placenta formation and organogenesis. Harmful factors in these periods can induce embryo mortality or different congenital anomalies (Hayes 1994).

Prior to implantation, the embryo is exposed to materials secreted by the epithelium of endometrium and Fallopian tubes. The compounds that primarily function as regulatory agents for embryo development and implantation comprise enzymes, cytokines, growth factors, ions, hormones, glucose, transport proteins, and adhesion molecules (Gray et al. 2001; Boomsma et al. 2009). This means that an exposure of embryos to NP before their implantation may be possible after NP pass to endometrial gland cells and when they are later secreted to the fetal environment.

In the post-implantation stages the embryo is surrounded by maternal tissues and becomes more dependant on maternal nutrition as the embryo grows. At the time, the maternal-fetal barrier is determined as a selective molecule transport from maternal blood across the trophoblast and vice versa. The main role in the biological barrier is attributed to the placenta. However, the formation of placenta starts after implantation and lasts during the first trimester (the term differs among species), therefore, the embryo is separated by a specific but still not fully mature barrier from mother's blood circulation during placentation. In this period, the trophoblast cells are invading into uterus tissue by internalizing endometrial cells and the tissue is being remodelled (Bevilacqua et al. 2010). During these changes there is a greater possibility for xenobiotics, including NP, to enter the fetal tissues what leads to a greater risk of possible effects on the development (Grazeliene et al. 2006).

When the placenta is completely formed, it becomes a natural barrier for a large variety of substances with diverse molecular structures, and the transport efficiency is regulated by the expression of cellular proteins (Marin et al. 2004). A mature placenta has several functions in embryogenesis because it contains cells that endocytize exogenous antibodies and produce various hormones and other regulatory molecules. It takes place in ion transport, liquid regulation, and transmits the information to the embryo (Gude et al. 2004; Sibley 2009). At this period the barrier properties are highly pronounced and the fetal nutrition is ensured for the proper development.

At the end of embryogenesis the placenta starts to reorganize before the birth. Capability of the barrier is changing: in rodents, the trabecular structures of the labyrinth alter by increasing the amount of the connective tissue, the calcification zones originate, the blood vessels are breaking (Metz 1980; Akirav et al. 2005). Human placenta also undergoes calcification and histological reorganization before the term (Chen et al. 2011). The structural changes highly influence the overall placental functions and may possibly increase the barrier permeability for NP.

The structure and function of the placental barrier

A placenta is a physiological connection between fetal and maternal tissues regulating the nutrition, respiration and excretion of the fetus. When the placenta is formed the molecules may pass to the embryonic blood via the placental barrier and the visceral yolk sac placenta, which is present in rodents. It is assumed that the placental barrier plays the major role in the maternal-fetal transfer of biomolecules. As the placental structure varies greatly among species we here represent the principal structure of hemochorial placenta in humans and rodents because these types of placenta are widely used in transplacental NP penetration experiments. The term placenta will be used.

In humans, the placenta is divided by decidual septa into separate functional vascular units known as cotyledons. Within each cotyledon, there is a chorionic villous tree structure which is rich in fetal capillaries. There are a few layers of extraembryonic membranes in the placental structure: fetal capillaries endothelium, connective tissue (chorioallantoic mesoderm) and the trophoblast which is formed by a continuous layer of syncytiotrophoblast and the underlying cytotrophoblast cells (Fig. 1). These layers form a chorionic villi which are exposed to maternal blood. Reorganization of the endometrium connective tissue and maternal capillaries during the placentation processes leads to maternal blood release into intervillous chamber which surrounds the chorionic villi and the molecules may be transported from blood across the syncytiotrophoblast cells and vice versa (Saunders 2009).

A rodent placenta is composed of a decidua, a junctional zone and a labyrinth (Fig. 2, A). The former is the area where the maternal and fetal components integrate and where exchange of nutrients takes place. It is characterized

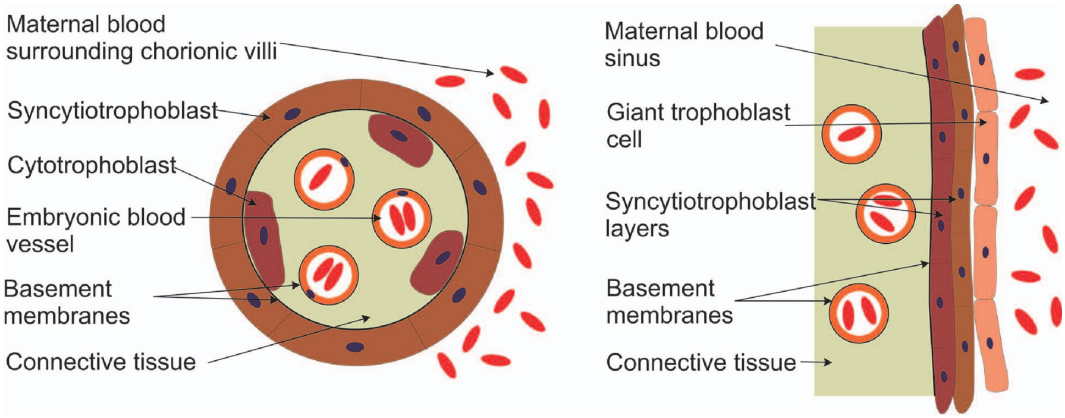

Fig. 1. Schematic representation of the placental barrier. In humans (left) the fetal connective tissue is separated from maternal blood by two trophoblast layers: the syncytiotrophoblast and the cytotrophoblast (a discontinuous layer). Meanwhile, in rodents (right) there are three continuous trophoblast layers. The fetal capillary endothelium and the underlying basement membrane are present in all mammals. In humans the chorion forms round-shaped villous structure, while in rodents the chorion and maternal sinuses form the labyrinth of irregular structure.

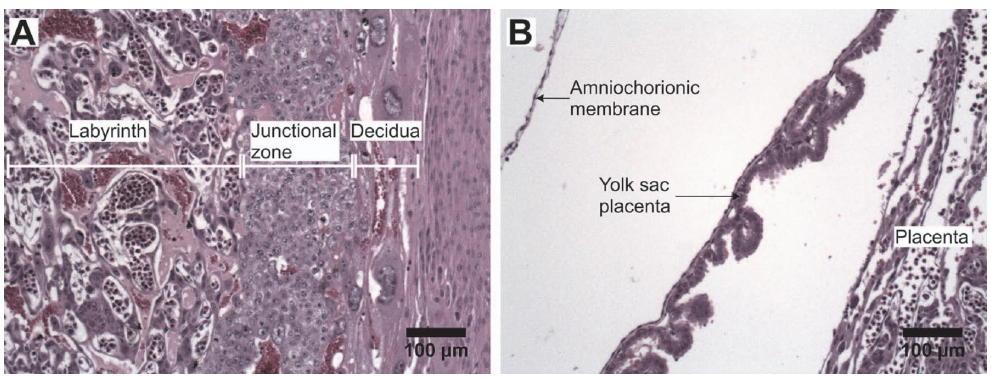

Fig. 2. Histological images of the placenta.
Shown are the histological images of rat placenta (A) and yolk sac placenta (B) on the day 13 of gestation (full term - 21 days). The tissues were prepared using standard formalin fixation and paraffin embedding techniques, sectioned with microtome and stained with haematoxylin - eosin. Images captured using Nikon Eclipse TE2000 microscope (× 10/0.25 objective) and Leica DFC290 camera. Scale bars - 100 μm.

by excessive branching of the fetal villi increasing the surface area for the exchange of nutrients. Rodent placentas are classified as hemotrichorial since the barrier is composed of three cellular layers: the cytotrophoblast layer (sometimes called mononuclear trophoblast giant cells) and two syncytiotrophoblast layers (Fig. 1) (Takata et al. 1997; Kibschull et al. 2008).

The placental barrier is mainly formed by cells exposed to the blood: trophoblast cells on the maternal side and capillary endothelium on the fetal side. These cell layers have characteristic structure which determines their interceptive properties: the basal surfaces of the endothelial and trophoblast cells are exposed to the basement membrane which organizes the cellular layer, and the lateral cellular sides of the endothelial cells are interconnected with tight junctions, adherence junctions and desmosomes. These junctional complexes completely encircle cells resulting in a continuous connecting belt that regulates paracellular permeability and prevents passage of macromolecules (Steed et al. 2010). In addition, the tight junctions impede protein intramembrane diffusion between the apical and basal cell poles resulting in asymmetrical protein distribution on the opposite endothelium sides. Consequently, it determines different molecule transport efficiency and different membrane functions on the opposite sides of the barrier. The syncytiotrophoblast cells form a continuous layer of syncytium which is maintained by a fusion process of the underlying cytotrophoblast cells (Kibschull et al. 2008). These structural properties ensure that large molecules pass to the embryonic tissues mainly by entering cells of the trophoblast and endothelium. The compounds might enter the cellular membrane via classical passive (facilitated diffusion, filtration, etc.) and active (carrier-mediated transport, endocytosis, etc.) transport systems. In both cases the molecule uptake is controlled by the expression of specific membrane proteins. For instance, the apical membrane of endothelial cells contains specific transporters for serotonin, carnitine or folate. It also has

nonspecific transporters such as P-glycoprotein, a member of the multidrug resistance mechanism (Wick et al. 2010). The active transport systems play a key role in the intracellular transport of macromolecules and NP. Extensive studies have shown that NP internalization mainly occurs via endocytotic pathways and depends on NP properties (e.g., size, charge, functionalization) as well as on the cellular origin and physiology (Kelf et al. 2010; Pi et al. 2010; Tan et al. 2010).

Some authors report presence of transtrophoblastic channels which are thought to be membrane-lined tubular systems that originate from membrane invaginations of the basal syncytiotrophoblastic surface and cross the cell towards its apical surface (Kertschanska et al. 1997; Benirschke and Kaufman 2000). They might function as pressure-dependent valves regulating fetal water and biomolecules transport by changing the effective molecular diameter in the range of 2-25 nm. However, the presence and physiological role of these channels is uncertain and remains disputed among scientists (Bonatelli et al. 2005; Menjoge et al. 2011).

The villous stroma located between fetal capillaries and the trophoblast layer also plays an important role in barrier physiology. It contains Hofbauer cells, which regulate placental vasculogenesis and which are typical macrophages with pinocytotic and phagocytotic activity (Seval et al. 2007).

The placenta in humans and rodents is called hemochorionic and due to its shape is sometimes named a discoid placenta. However, rodents additionally have a visceral yolk sac placenta (choriovitelline placenta) (Fig. 2, B). It forms continuous fetal coating separating vitelline capillaries from the maternal endometrium and it plays an important role in the nutrition during the early embryogenesis stages (Farese et al. 1996). It is said to be "inverted" (the absorptive surface is exposed to the maternal tissues rather than to the yolk) and it absorbs molecules and transports them into the embryonic circulation (cholesterol, ions, amino acids, immunoglobulins etc.) (Hatzopoulos et al. 1998; Freyer and Renfree 2009). In mice, this transport is thought to provide materials to the embryo until at least day 10 of the 20-21 total pregnancy days (Farese et al. 1996). Therefore the yolk sac placenta could be treated as a possible NP transfer pathway. The yolk sac membrane is formed basically of three layers: an outer endodermal epithelium, an intermediate mesenchymal tissue within which blood vessels develop and an inner mesothelial epithelium (El-Nefiawy et al. 2002). In contrast to the blood sinuses of the hemochorionic placenta, the visceral yolk sac placenta absorbs molecules from the intact endometrial capillaries, and therefore molecules or NP have to pass the endothelium barrier before reaching fetal tissues. The basement membrane of maternal endothelial cells is called Reichert's membrane and it also plays a significant role in molecule transport (Farese et al. 1996). In general, the visceral yolk sac placental barrier is constituted from these layers: maternal vessels endothelium, trophoblast cells, Reichert's membrane, visceral yolk sac epithelium and fetal vessels endothelium (Hatzopoulos et al. 1998).

It should be noted that the physiology and functionality of the maternal-fetal barrier change during the embryogenesis, and the maternal NP exposure in different pregnancy stages may result in different biological effects upon the fetus development.

In vivo studies of transplacental passage of nanoparticles

By now, there are only few in vivo studies on NP penetration through a hemochorial placental barrier. Recently Chu et al. (2010) reported about semiconductor NP penetration across the placental barrier and their accumulation in mice embryos. The authors used quantum dots (QD) composed of a CdTe core, covered with mercaptopropionic acid (MPA). The nanoparticles were injected into the tail vein of pregnant mice 1-5 days before giving the birth, when the placental barrier was completely developed. Cd accumulation in embryos reached up to 0.6% of the injected dose (20 μg of Cd). The authors established that NP transplacental penetration depends on the size and coating of QD. Application of smaller QDs (1.7 nm compared to 2.6 and 3.2 nm) resulted in higher Cd levels. When the particles were stabilized with a SiO_2 or polyethylene glycol (PEG) coating, the transfer decreased.

However, it should be noted that the quantitative QD distribution analysis was carried out by means of inductive coupled plasma atomic emission spectrometry (ICP-AES)—a technique which is sensitive to elemental composition, particularly, to cadmium. It means that the authors were unable to determine if the Cd in the embryos originated from Cd forming QD crystals or from Cd^{2+} ions which were released after QD degradation in the maternal organism. This ambiguity undermines the reliability of the ICP-AES method for the assessment of NP concentration in vivo. CdTe QD degradation in vivo and toxicity due to Cd^{2+} leakage are possible and the fact was reported earlier (Liu et al. 2008; Lin et al. 2009). Consequently, lower Cd accumulation after coating the nanoparticles can be explained in two ways: a) the coating stabilizes the CdTe core and minimizes Cd^{2+} release; b) the coating increases the overall NP size, which results in reduced penetration through placental barrier. It is worth mentioning that non-coated CdTe-MPA QDs possess a negative charge, and that such nanoparticles interact with plasma proteins resulting in a significantly increased hydrodynamic diameter (Chen et al. 2008). As the PEG coating has a neutral charge, it diminishes protein adsorption and the consequential diameter growth. Therefore, the PEG coating may affect the NP diameter in vivo in two opposite ways and additional quantitative studies necessary to determine the coating effect on NP penetration through the placental barrier.

The authors also observed embryotoxicity after a QD injection. The toxic effect was lower using QD of a larger

diameter and using SiO_2 or PEG coatings. In general, embryotoxicity correlated with cadmium concentration in embryos.

A recent study of another group also reported pregnancy complications in mice induced by NP (Yamashita et al. 2011). The authors examined the distribution and fetal toxicity of differently sized silica and titanium oxide (TiO_2) NP. The whole-body optical imaging analysis showed accumulation of fluorescently labelled 70 nm size silica and 143 nm size TiO_2 NP in the placenta 24 hours after an intravenous NP injection. Electron microscopy revealed that these NP were localized in placental trophoblast cells, as well as in the fetal liver and fetal brain. However, silica NP of a bigger size, 300 nm or 1,000 nm, were not observed in placenta or fetuses. The authors speculate that NP penetrated through the placenta by active transcellular transport or directly injured the placental barrier. The NP appearance in fetal brain is explained by an incompletely formed blood-brain barrier in the current embryogenesis stage. Experiments have shown that NP induced adverse effects on embryogenesis including growth inhibition, resorptions, placental dysfunction and other functional and structural changes. The provoked physiological changes correlate with the nanoparticles penetration across the barrier and they were not observed for the 300 nm and 1,000 nm NP which did not accumulate in the placenta and fetuses. NP toxicity depended on NP concentration and it could be prevented with coating the silica NP surface with carboxy or amino groups. Interestingly, the NP accumulation in fetuses did not change after coupling the surface ligands.

The toxicity was also assessed for the fullerene NP of the size of 65-143 nm, but no adverse effects were observed. These results suggest that the fullerene NP could not pass into the embryos due to their size when compared to silica NP of similar size which crossed the placental barrier. Unfortunately, the absence of fullerene toxicity was not discussed in the report, but the distinct biological effect could be determined by the differences in NP surface and chemical composition when compared with the toxic silica and TiO_2 NP.

Another group investigated the penetration of gold nanoparticles through a rat placenta (Semmler-Behnke et al. 2007). NP of two sizes (1.4 nm and 18 nm) were radio labelled and stabilized by negatively charged ionic ligands. At 24 hours after intravenous injection of gold NP, they found a rather strong and inversely size-dependent NP uptake in the placenta: about 3% of 1.4 nm NP and about 0.02% of 18 nm NP. Respectively, the concentration in the fetuses was also higher for smaller NP. Biological effects of NP exposition on embryogenesis were not studied.

The discussed reports show that NP penetration across the placental barrier depends on the size and chemical composition of the surface coating of NP. However, the studies were carried out under different experimental conditions; therefore it is difficult to determine the size threshold for maternal-fetal NP transfer. The results show that physico-chemical properties of NP highly depend on the surface coating. The surface properties determine the overall stability of the NP, the interaction with biomolecules and the distribution in the organism. The experimental studies on rodents are meaningful as they provide principal knowledge about distribution and physiological effects of NP during pregnancy. The results of the research in NP transplacental penetration has a lasting legacy for possible veterinary applications as well as for understanding fundamental processes which determine NP passage through biological barriers. However, the differences in placental structure among species limit the extrapolation of the results of animal studies for humans and hinder their applications for the development of diagnostic or therapeutic techniques. Thus, the studies using ex vivo human placentas have additional advantage as they model the processes taking place after NP exposure during women pregnancy.

Ex vivo studies in transplacental passage of nanoparticles

Myllynen et al. (2008) investigated penetration of gold nanoparticles through the placenta barrier using an ex vivo human placenta perfusion model. Monodispersed NP of the size of 10, 15 and 30 nm were coated with PEG and perfused for 6 hours. However, gold NP of any size were not observed in the fetal part of the placenta. After the perfusions, the NP concentration in the maternal samples decreased up to 64% of the initial dose suggesting that particles were taken up into tissues. An electron microscopy analysis confirmed that NP mainly localized in the syncytiotrophoblast and trophoblast layers, but not in the fetal capillary endothelium, confirming that human placental barrier is impermeable for the PEG coated gold NP.

Wick et al. (2010) employed fluorescently labelled polystyrene beads with diameters of 50-500 nm to investigate the size-dependent NP passage in the ex vivo human placental perfusion model. The results showed that beads up to the size of 240 nm were taken up in placenta, and subsequently crossed the placental barrier without affecting viability of the tissues. The major possible mechanisms of NP transfer involve diffusion and vesicular transport such as clathrin- or caveolin-mediated endocytosis peculiar for smaller particles. Meanwhile, these types of endocytosis are unlikely for 240 nm spheres leaving the question of NP transport mechanism unanswered.

The ex vivo human placenta perfusion model was also employed for the assessment of polyamidoamine (PAMAM) dendrimers penetration to the fetus (Menjoge et al. 2011). The diameter of fluorescently labelled NP was in the range of 5-6 nm. The HPLC analysis revealed that dendrimers could be first detected in the fetal perfusate after 15 min and the concentration was increasing during the whole experiment which lasted up to 5.5 hours. The final dendrimers concentration ratio in fetal maternal compartment reached the value of 0.07 and the absolute NP concentration in fetal compartment constituted ~3% of initial dose in

maternal perfusate. The fluorescence microscopy tissue analysis revealed that dendrimers mainly accumulated in the inter-villous space surrounding the rims of the syncytiotrophoblast. In individual cases, the NP were observed inside the trophoblast cells and the villous core but not in the fetal capillaries. However, the microscopy resolution is far too low to resolve single nanoparticles and the fluorescence signal reflects the fact that dendrimers are present in the region. The results indicate that NP used in the experiments are not retained to a significant degree in a human placenta. According to the authors, the NP transfer is mainly attributed to the paracellular diffusion due to the small size of NP (5.5 nm) and the transcellular transport is assumed as being less likely. The results also indicate that drug-dendrimers conjugates would be restricted from passing across the human placenta when compared to small drugs alone.

To sum up the discussed studies, it can be concluded that NP transplacental passage is size depended. The proposed transport mechanisms are different for NP of distinct size ranges: paracellular diffusion dominates in the case of small (~5.5 nm) NP (Menjoge et al. 2011) and transcellular transfer is more likely for larger (50-240 nm) NP (Wick et al. 2010). However the penetration pathways are still hypothetical and experimentally unproven. The different results of Myllynen et al. (2008) and Wick et al. (2010), who used NP of similar sizes, might be attributed to the different surface coatings of the NP. The variation in experimental conditions and NP stability may also contribute to these differences. The performed ex vivo models have certain disadvantages over in vivo studies. The perfusion time is limited to a few hours because of tissue degradation. The explants represent only the late phase of pregnancy for which the barrier thickness is reduced, and the placenta undergoes significant remodelling before giving the birth. Therefore, the barrier permeability is possibly increased at the term when compared to the earlier embryogenesis stages. It should be noted that NP accumulation in the trophoblast (Myllynen et al. 2008; Menjoge et al. 2011) might induce long-term physiological effects on the cellular activity and result in slow NP release to the fetal compartment. These questions were out of the scope in current studies and they are difficult to elucidate using ex vivo techniques. Therefore, the ex vivo models might not fully represent the NP distribution, fetal accumulation and the subsequent physiological effects after maternal exposure to NP during pregnancy.

The possible mechanisms of transplacental transport of NP

The discussed studies show that NP transplacental passage occurs under different experimental conditions, particularly using in vivo rodent and ex vivo human perfusion models. The reports indicate that NP transport highly depends on NP size. The threshold of NP penetration across the barrier was found to be between 143-300 nm for silica NP in mice (Yamashita et al. 2011) and between 80-240 nm for polystyrene NP in humans (Wick et al. 2010). The size-dependent NP penetration was also determined for other barriers, e.g. for the particle filtration in kidneys. However, different authors indicate that the glomerular filtration threshold for NP is between 6-8 nm (Choi et al. 2007; Longmire et al. 2008), which is significantly lower than that reported for placental barrier. In addition, the trophoblast cells are interconnected and form a continuous layer of syncytium, minimizing NP interception between the cells (Kibschull et al. 2008). Therefore, the paracellular NP diffusion across the trophoblast is unlikely and might be neglected as an NP passage pathway for NP larger than 10 nm. Theoretically, some NP could pass the trophoblast layer via the transtrophoblastic channels, which might dilute up to 20-25 nm in diameter in the case of appropriate hydrostatic pressure (Menjoge et al. 2011). Still, the performed electron microscopy and confocal microscopy studies have not confirmed the presence of such pathway. Nevertheless, the large NP used in the current reports have to be absorbed inside the trophoblast cells to be afterwards transferred to the fetal circulation. It is assumed that NP mainly enter the cells by the active transport, particularly, via the endocytotic pathways (Pi et al. 2010; Tan et al. 2010).

The transplacental NP uptake was observed despite different intrinsic chemical composition of the particles (CdTe, polymers, silica, TiO_2, gold), whereas surface coating had significant impact on NP distribution and physiological effects. Interestingly, only one study showed NP sequestration in the placental tissue without passing into the fetus: PEG coated 10-30 nm gold nanoparticles (Myllynen et al. 2008). Since the NP were modified with a polymer, the size might have increased and the final diameter of the modified NP was undetermined. The influence of NP surface on the interaction with biomolecules and intracellular uptake was investigated in vitro (Tan et al. 2010) and in vivo (Choi et al. 2007) earlier. The studies showed that negatively charged particles are better endocytized by endothelial vessel cells (Praetner et al. 2010). On the other hand, positively charged NP proved to be more efficiently filtrated in kidneys (Longmire et al. 2008). Neutral NP possess lower protein adhesion, less efficient cellular uptake and longer circulation times in blood (Mosqueira et al. 2001). NP hydrophilicity also has a significant effect on endocytotic efficiency (Tan et al. 2010). Similarly to these effects, the chemical composition of NP surface should determine NP interaction with the receptors of placental trophoblast cells and, consequently, influence the efficiency of active transport to the fetal capillaries.

The events leading to particle engulfment and internalization are extremely complex. It starts after the particle adherence to the cellular membrane. This interaction is associated with different types of receptors like those for the component 3 (C3R) of the complement system, Fc region of immunoglobulins (FcR), mannose and many oth-

ers. In general, endocytosis can be dramatically enhanced when, under certain circumstances, the particles are coated (opsonized) by specific ligands having affinity for the cellular receptors, e.g. IgG, C3b, fibronectin or opsonins (Bevilacqua et al. 2010). Experiments show that various opsonizing proteins mark NP for intracellular uptake by the mononuclear phagocytic system determining the NP interactions, clearance, and biodistribution. Polar NP coatings result in higher opsonization when compared with a neutrally charged surface. For example a coating with PEG largely prevents protein binding and thus renders them invisible for macrophages (Choi et al. 2007; Rehberg et al. 2010). Although PEG is often used to make the NP inert to biological environment, it doesn't prevent interactions with all biomolecules. On the contrary, PEG coated quantum dots (QD) were reported to form clusters with lipid structures at the vessel wall in vivo (Rehberg et al. 2010) and intracellular lipid droplets in human epidermal keratinocytes in vitro (Ryman-Rasmussen et al. 2006). PEG is able to induce fusion of lipids like phosphatidylcholine in a concentration dependent manner (Boni et al. 1981) and therefore affect the biological membranes. The NP clusterization might impede their passage through a placental barrier due to an increase in the particle size. A reduced NP transplacental penetration was observed after coating QD with PEG in vivo (Chu et al. 2010), and the transfer was totally prevented for PEG coated gold NP in vitro (Myllynen et al. 2008).

Phagocytosis is also an important process for trophoblast nutrition by engulfing large objects. Therefore, it might significantly contribute to NP uptake and complement other endocytotic uptake pathways. The phagocytotic trophoblast activity was demonstrated in the pre-implantational embryogenesis stages using latex particles (1-3 μm) (Rassoulzadegan et al. 2000). This process is also of crucial importance during placentation as the trophoblast cells completely embrace and internalize uterine epithelial cells occupying the uterine territories. The phagocytotic activity lasts in the later stages as it executes erythrocyte internalization which is directly related to protein and iron transfer to the fetus. These facts indicate that NP phagocytosis can occur at different stages of embryogenesis and should be more significant for transplacental passage of larger NP (up to several μm sizes) and their clusters.

It should be noted that the yolk sac placenta in rodents also plays a significant role in material exchange with fetus, especially in earlier embryogenesis phases before a placental circulation is formed (Farese et al. 1996). Yolk sac placenta has the multiligand receptor complexes that are important for endocytotic pathways. These receptors are responsible for the absorption of many nutrients and, therefore, they could play an important role in NP passage as well (Zohn and Sarkar 2010). Unfortunately, by now there are no studies on NP passage across the yolk sac placenta.

Table 1. Summary of reports about NP effects on embryogenesis.

Nanoparticles	Model	Effects on embryogenesis	Reference
CdTe quantum dots with mercaptopropionic acid, SiO_2 or PEG coatings.	Mice / intravenous injection in vivo	Toxicity. The effect depends on NP size and coating. In general, toxicity correlates with Cd accumulation in fetus.	Chu et al. 2010
TiO_2 NP (35 nm) Silica NP (70-1,000 nm)	Mice/ intravenous injection in vivo	Toxicity as well as structural and functional changes in the placenta. The effects depend on the type and the surface coatings of the NP.	Yamashita et al. 2011
TiO_2 NP coated with polyalcohols (21 nm)	Mice/ powder inhalations in vivo	Neurobehavioral alterations without NP accumulation in fetus. No changes.	Hougaard et al. 2010
TiO_2 NP (2,570 nm)	Mice/ subcutaneous injection in vivo	Altered fetal gene expression associated with brain development, cell death, oxidative stress and inflammation. The effects depend on the duration of exposure and gestation period.	Shimizu et al. 009
CdSe and CdSe/ZnS quantum dots with mercaptoacetic acid coating (2-4 nm).	Mice oocytes/ in vitro	Toxicity on oocyte maturation, fertilization and embryogenesis. QD induce oocyte apoptosis and inhibit postimplantational development. The toxicity depends on NP coating. Possible teratogenic effects.	Hsieh et al. 2009
MgO NP (100 nm)	Human umbilical vein endothelial cells/ in vitro	Dose dependent cytotoxicity	Ge et al. 2011
CdTe quantum dots		Dose dependent cytotoxicity and genotoxicity (growth inhibition, ROS, DNA damage)	Wang et al. 2010
Carbon nanotubes (30 nm)		Cytotoxicity and genotoxicity (reduced viability, induced apoptosis, ROS, DNA damage)	Guo et al. 2011
CdSe/ZnS quantum dots with PEG or poly-lysine coatings (7-14 nm)	zebrafish larvae/in vitro	Toxicity. The effect depends on NP size and coating. The QD exposure leads to Cd toxicity endpoints and additional effects (distinct from Cd) like necrosis, yolk sac and tail malformations.	King-Heiden et al. 2009

Effects of nanoparticles on embryogenesis

The discussed NP passage to the embryos raises concerns about possible toxic and teratogenic effects on fetal development. Recent reports about an adverse impact on embryogenesis after a maternal exposure of NP are presented in Table 1. By now, there have been no data on NP toxicity on human embryos, and most in vivo studies have been made using rodent models. Scrutiny in the observed NP effects leads to a conclusion that different experimental conditions induced embryotoxicity, which depends on physicochemical NP properties such as size and surface coating. NP of smaller sizes penetrate the embryos easier and accumulate in higher quantities. The surface stabilization with SiO2, ZnS, PEG or other organic ligands (King-Heiden et al. 2009; Hsieh et al. 2009; Chu et al. 2010; Yamashita et al. 2011) also reduces the transplacental transfer. NP toxicity mechanisms have not been well established yet. Moreover, they depend on experimental conditions, the route of administration and NP type. However, recent studies imply that NP toxicity might be mediated by oxidative stress and inflammation (Shimizu et al. 2009; Wang et al. 2010), cell growth inhibition, enhanced apoptotic processes (Hsieh et al. 2009; Guo et al. 2011), necrosis (King-Heiden et al. 2009), and/or DNA damage (Wang et al. 2010).

In the case of quantum dots (QD) the toxicity is related with the release of Cd^{2+} ions and subsequent Cd^{2+} induced effects (Karabanovas et al. 2008; Hsieh et al. 2009). However King-Heiden et al. (2009) observed QD induced necrosis and yolk sac and tail malformation, which were not characteristic to Cd^{2+} evoked effects, indicating that nanostructure exhibits new qualities that are not prominent in their components. Hougaard et al. (2010) observed adverse effects on neurobehavior and fertility of the offspring's of TiO_2 exposed rats. The research highlighted that the maternal NP exposure can lead to indirect effects on embryogenesis without NP accumulation in the fetus. This possibility was corroborated by observations of changes in the expression of genes associated with acute phase, inflammation and immune response.

Finally, it should be noted that interests in physiological effects of NP during pregnancy are growing and the number of scientific studies is increasing. NP accumulation in embryos and the subsequent beneficial or adverse effects to the organism depend on the type and physicochemical properties of NP as well as dosage, route of administration, gestation period, duration of exposure and other experimental conditions. Since different research groups use individual models, there is a need of experimental standardization in order to compare different studies and to obtain systemic knowledge about safety of using NP in industry and medicine. Researches on human models are of great demand because the placental barrier structure, immune responses and the physiology of embryogenesis is different from that of experimental animals, what limits the extrapolation of the results to human embryogenesis. The human placental perfusion model was successfully used in NP transplacental transfer studies and the experimental conditions can be sufficiently maintained to obtain reproducible results, therefore the placental perfusion model could be standardized for the assessment of NP transplacental passage. However placental barrier physiology highly depends on the gestation period (Gude et al. 2004; Shimizu et al. 2009) what raises the need for other experimental models that would produce data on NP passage in early stages of embryogenesis.

Acknowledgments

This research was funded by the grant (No. MIP-10440) of the Research Council of Lithuania and by the grant (No. 2004-LT0036-IP-1NOR) of Norwegian financial mechanism.

Conflicts of Interest

All authors of this study have no conflicts of interest regarding this paper.

References

Adair, B.M. (2009) Nanoparticle vaccines against respiratory viruses. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, **1**, 405-414.

Akirav, C., Lu, Y., Mu, J., Qu, D.W., Zhou, Y.Q., Slevin, J., Holmyard, D., Foster, F.S. & Adamson, S.L. (2005) Ultrasonic detection and developmental changes in calcification of the placenta during normal pregnancy in mice. *Placenta*, **26**, 129-137.

Benirschke, K. & Kaufmann, P. (2000) *Pathology of the human placenta,* 4th ed., Springer-Verlag, New York, NY.

Bevilacqua, E., Hoshida, M.S., Amarante-Paffaro, A., Albieri-Borges, A. & Zago Gomes, S. (2010) Trophoblast phagocytic program: roles in different placental systems. *Int. J. Dev. Biol.*, **54**, 495-505.

Bonatelli, M., Carter, A.M., Machado, M.R., De Oliveira, M.F., de Lima, M.C. & Miglino, M.A. (2005) Placentation in the paca (Agouti paca L). *Reprod. Biol. Endocrinol.*, **3**, 9.

Boni, L.T., Stewart, T.P., Alderfer, J.L. & Hui, S.W. (1981) Lipid-polyethylene glycol interactions: Induction of fusion between liposomes. *J. Membr. Biol.*, **62**, 65-70.

Boomsma, C.M., Kavelaars, A., Eijkemans, M.J., Lentjes, E.G., Fauser, B.C., Heijnen, C.J. & Macklon, N.S. (2009) Endometrial secretion analysis identifies a cytokine profile predictive of pregnancy in IVF. *Hum. Reprod.*, **24**, 1427-1435.

Chen, K.H., Chen, L.R. & Lee, Y.H. (2011) Exploring the relationship between preterm placental calcification and adverse maternal and fetal outcome. *Ultrasound Obstet. Gynecol.*, **37**, 328-334.

Chen, Z., Chen, H., Meng, H., Xing, G., Gao, X., Sun, B., Shi, X., Yuan, H., Zhang, C., Liu, R., Zhao, F., Zhao, Y. & Fang, X. (2008) Bio-distribution and metabolic paths of silica coated CdSeS quantum dots. *Toxicol. Appl. Pharmacol.*, **230**, 364-371.

Choi, H.S., Liu, W., Misra, P., Tanaka, E., Zimmer, J.P., Itty Ipe, B., Bawendi, M.G. & Frangioni, J.V. (2007) Renal clearance of quantum dots. *Nat. Biotechnol.*, **25**, 1165-1170.

Chu, M., Wu, Q., Yang, H., Yuan, R., Hou, S., Yang, Y., Zou, Y., Xu, S., Xu, K., Ji, A. & Sheng, L. (2010) Transfer of quantum dots from pregnant mice to pups across the placental barrier. *Small*, **6**, 670-678.

El-Nefiawy, N., Abdel-Hakim, K., Yamashita, A., Kanayama, N. (2002) Embryonic macrophages of early rat yolk sac: Immunohistochemistry and ultrastructure with reference to endo-

dermal cell layer. *Immunol. Cell Biol.*, **80**, 441-447.
Farese, R.V. Jr., Cases, S., Ruland, S.L., Kayden, H.J., Wong, J.S., Young, S.G. & Hamilton, R.L. (1996) A novel function for apolipoprotein B: lipoprotein synthesis in the yolk sac is critical for maternal-fetal lipid transport in mice. *J. Lipid Res.*, **37**, 347-360.
Freyer, C. & Renfree, M.B. (2009) The mammalian yolk sac placenta. *J. Exp. Zool. B. Mol. Dev. Evol.*, **312**, 545-554.
Ge, S., Wang, G., Shen, Y., Zhang, Q., Jia, D., Wang, H., Dong, Q. & Yin, T. (2011) Cytotoxic effects of MgO nanoparticles on human umbilical vein endothelial cells in vitro. *IET Nanobiotechnol.*, **5**, 36.
Gray, C.A., Taylor, K.M., Ramsey, W.S., Hill, J.R., Bazer, F.W., Bartol, F.F. & Spencer, T.E. (2001) Endometrial glands are required for preimplantation conceptus elongation and survival. *Biol. Reprod.*, **64**, 1608-1613.
Grazeliene, G., Zalgeviciene, V., Didziapetriene, J., Zukiene, J., Sukackaite, A., Legenis, V. & Rotomskis, R. (2006) Accumulation of photosensitizer in rat embryos (a spectroscopic study). *Medicina (Kaunas),* **42**, 142-146.
Gude, N.M., Roberts, C.T., Kalionis, B. & King, R.G. (2004) Growth and function of the normal human placenta. *Thromb. Res.,* **114**, 397-407.
Guo, Y.Y., Zhang, J., Zheng, Y.F., Yang, J. & Zhu, X.Q. (2011) Cytotoxic and genotoxic effects of multi-wall carbon nanotubes on human umbilical vein endothelial cells in vitro. *Mutat. Res.*, **721**, 184-191.
Gupta, R.C. (2007) *Veterinary toxicology,* 1st ed., Academic Press (Elsevier), New York, NY.
Hardman, R. (2006) A toxicologic review of quantum dots: toxicity depends on physicochemical and environmental factors. *Environ. Health Perspect.*, **114**, 165-172.
Hatzopoulos, A.K., Rigotti, A., Rosenberg, R.D., Krieger, M. (1998) Temporal and spatial pattern of expression of the HDL receptor SR-BI during murine embryogenesis. *J. Lipid Res.*, **39**, 495-508.
Hayes, A.W. (1994) *Principles and Methods of Toxicology*, Raven Press, New York, NY. P. 1006-1036.
Hougaard, K.S., Jackson, P., Jensen, K.A., Sloth, J.J., Löschner, K., Larsen, E.H., Birkedal, R.K., Vibenholt, A., Boisen, A.M., Wallin, H. & Vogel, U. (2010) Effects of prenatal exposure to surface-coated nanosized titanium dioxide (UV-Titan). A study in mice. *Part Fibre Toxicol.*, **7**, 16.
Hsieh, M.S., Shiao, N.H. & Chan, W.H. (2009) Cytotoxic effects of CdSe quantum dots on maturation of mouse oocytes, fertilization, and fetal development. *Int. J. Mol. Sci.*, **10**, 2122-2135.
Karabanovas, V., Zakarevicius, E., Sukackaite, A., Streckyte, G. & Rotomskis, R. (2008) Examination of the stability of hydrophobic (CdSe)ZnS quantum dots in the digestive tract of rats. *Photochem. Photobiol. Sci.,* **7**, 725-729.
Kelf, T.A., Sreenivasan, V.K.A., Sun, J., Kim, E.J., Goldys, E.M. & Zvyagin, A.V. (2010) Non-specific cellular uptake of surface-functionalized quantum dots. *Nanotechnology*, **21**, 285105.
Kertschanska, S., Kosanke, G. & Kaufmann, P. (1997) Pressure dependence of so-called transtrophoblastic channels during fetal perfusion of human placental villi. *Microsc. Res. Tech.*, **38**, 52-62.
Kibschull, M., Gellhaus, A. & Winterhager, E. (2008) Analogous and unique functions of connexins in mouse and human placental development. *Placenta*, **29**, 848-854.
King-Heiden, T.C., Wiecinski, P.N., Mangham, A.N., Metz, K.M., Nesbit, D., Pedersen, J.A., Hamers, R.J., Heideman, W. & Peterson, R.E. (2009) Quantum dot nanotoxicity assessment using the zebrafish embryo. *Environ. Sci. Technol.*, **43**, 1605-1611.
Lin, C.H., Chang, L.W., Chang, H., Yang, M.H., Yang, C.S., Lai, W.H., Chang, W.H. & Lin, P. (2009) The chemical fate of the Cd/Se/Te-based quantum dot 705 in the biological system: toxicity implications. *Nanotechnology*, **20**, 215101.
Liu, L., Zhang, J., Su, X. & Mason, R.P. (2008) In vitro and In vivo Assessment of CdTe and CdHgTe Toxicity and Clearance. *J. Biomed. Nanotechnol.*, **4**, 524-528.
Longmire, M., Choyke, P.L. & Kobayashi, H. (2008) Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. *Nanomedicine (Lond)*, **3**, 703-717.
Marin, J.J., Briz, O. & Serrano, M.A. (2004) A review on the molecular mechanisms involved in the placental barrier for drugs. *Curr. Drug Deliv.*, **1**, 275-289.
Menjoge, A.R., Rinderknecht, A.L., Navath, R.S., Faridnia, M., Kim, C.J., Romero, R., Miller, R.K. & Kannan, R.M. (2011) Transfer of PAMAM dendrimers across human placenta: Prospects of its use as drug carrier during pregnancy. *J. Control Release*, **150**, 326-338.
Metz, J. (1980) On the developing rat placenta. I. Differentiation and junctional alterations of labyrinthine layers II and III. *Anat. Embryol. (Berl)*, **159**, 289-305.
Mosqueira, V.C., Legrand, P., Morgat, J.L., Vert, M., Mysiakine, E., Gref, R., Devissaguet, J.P. & Barratt, G. (2001) Biodistribution of long-circulating PEG-grafted nanocapsules in mice: effects of PEG chain length and density. *Pharm. Res.*, **18**, 1411-1419.
Myllynen, P.K., Loughran, M.J., Howard, C.V., Sormunen, R., Walsh, A.A. & Vähäkangas, K.H. (2008) Kinetics of gold nanoparticles in the human placenta. *Reprod. Toxicol.*, **26**, 130-137.
Pi, Q.M., Zhang, W.J., Zhou, G.D., Liu, W. & Cao, Y. (2010) Degradation or excretion of quantum dots in mouse embryonic stem cells. *BMC Biotechnology*, **10**, 36.
Praetner, M., Rehberg, M., Bihari, P., Lerchenberger, M., Uhl, B., Holzer, M., Eichhorn, M.E., Fürst, R., Perisic, T., Reichel, C.A., Welsch, U. & Krombach, F. (2010) The contribution of the capillary endothelium to blood clearance and tissue deposition of anionic quantum dots in vivo. *Biomaterials*, **31**, 6692-6700.
Rassoulzadegan, M., Rosen, B.S., Gillot, I. & Cuzin, F. (2000) Phagocytosis reveals a reversible differentiated state early in the development of the mouse embryo. *EMBO J.*, **19**, 3295-3303.
Rehberg, M., Praetner, M., Leite, C.F., Reichel, C.A., Bihari, P., Mildner, K., Duhr, S., Zeuschner, D. & Krombach, F. (2010) Quantum dots modulate leukocyte adhesion and transmigration depending on their surface modification. *Nano Lett.*, **10**, 3656-3664.
Ryman-Rasmussen, J.P., Riviere, J.E. & Monteiro-Riviere, N.A. (2006) Penetration of intact skin by quantum dots with diverse physicochemical properties. *Toxicol. Sci.*, **91**, 159-165.
Saunders, M. (2009) Transplacental transport of nanomaterials. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, **1**, 671-684.
Semmler-Behnke, M., Fertsch, S., Schmid, G., Wenk, A. & Kreyling, W. (2007) Uptake of 1.4 nm Versus 18 nm Gold Particles by Secondary Target Organs is Size Dependent in Control and Pregnant Rats After Intratracheal or Intravenous Application. *EuroNanoForum*. Dusseldorf: European Commission, 102-104.
Seval, Y., Korgun, E.T. & Demir, R. (2007) Hofbauer cells in early human placenta: possible implications in vasculogenesis and angiogenesis. *Placenta*, **28**, 841-845.
Shimizu, M., Tainaka, H., Oba, T., Mizuo, K., Umezawa, M. & Takeda, K. (2009) Maternal exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression related to braindevelopment in the mouse. *Part Fibre Toxicol.*, **6**, 20.
Sibley, CP. (2009) Understanding placental nutrient transfer-why bother? New biomarkers of fetal growth. *J. Physiol.*, **587**,

3431-3440.
Steed, E., Balda, M.S. & Matter, K. (2010) Dynamics and functions of tight junctions. *Trends Cell. Biol.*, **20**, 142-149.
Takata, K., Fujikura, K. & Shin, B. (1997) Ultrastructure of the Rodent Placental Labyrinth: A Site of Barrier and Transport. *J. Reprod. Dev.*, **43**, 13-24.
Tan, S.J., Jana, N.R., Gao, S., Patra, P.K. & Ying, J.Y. (2010) Surface-Ligand-Dependent Cellular Interaction, Subcellular Localization, and Cytotoxicity of Polymer-Coated Quantum Dots. *Chem. Mater.*, **22**, 2239-2247.
Wang, L., Zhang, J., Zheng, Y., Yang, J., Zhang, Q. & Zhu, X. (2010) Bioeffects of CdTe quantum dots on human umbilical vein endothelial cells. *J. Nanosci. Nanotechnol.*, **10**, 8591-8596.
Wick, P., Malek, A., Manser, P., Meili, D., Maeder-Althaus, X., Diener, L., Diener, P.A., Zisch, A., Krug, H.F. & von Mandach, U. (2010) Barrier capacity of human placenta for nanosized materials. *Environ. Health Perspect.*, **118**, 432-436.
Yamashita, K., Yoshioka, Y., Higashisaka, K., Mimura, K., Morishita, Y., Nozaki, M., Yoshida, T., Ogura, T., Nabeshi, H., Nagano, K., Abe, Y., Kamada, H., Monobe, Y., Imazawa, T., Aoshima, H., Shishido, K., Kawai, Y., Mayumi, T., Tsunoda, S., Itoh, N., Yoshikawa, T., Yanagihara, I., Saito, S. & Tsutsumi, Y. (2011) Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nat. Nanotechnol.*, **6**, 321-328.
Zohn, I.E. & Sarkar, A.A. (2010) The visceral yolk sac endoderm provides for absorption of nutrients to the embryo during neurulation. *Birth Defects Res. A. Clin. Mol. Teratol.*, **88**, 593-600.